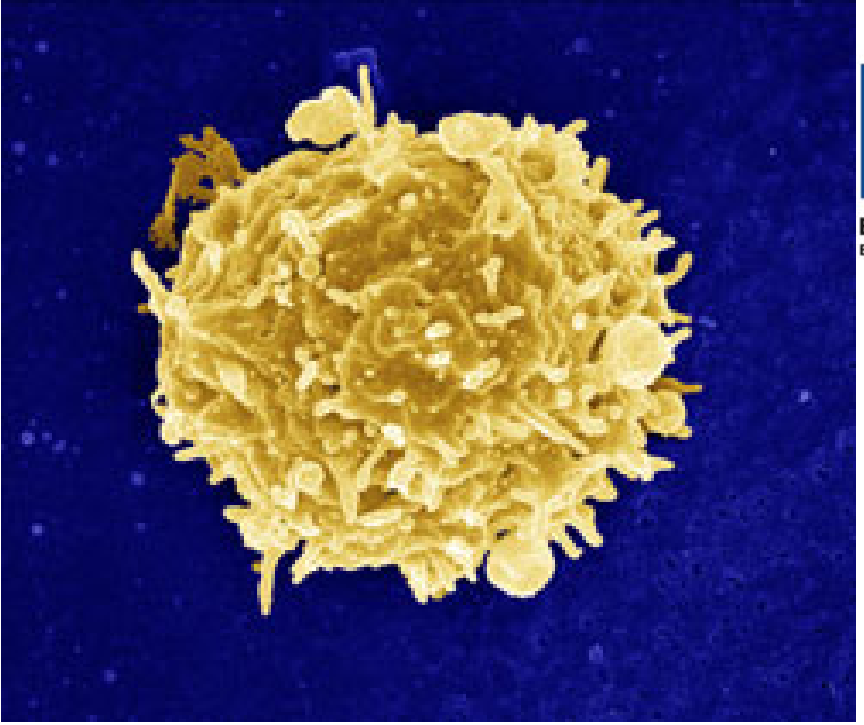


Ευρωπαϊκή Ένωση
Ευρωπαϊκό Κοινωνικό Ταμείο
ΕΠΙΧΕΙΡΗΣΙΑΚΟ ΠΡΟΓΡΑΜΜΑ
ΕΚΠΑΙΔΕΥΣΗ ΚΑΙ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗ
επένδυση στην κοινωνία της γνώσης
ΥΠΟΥΡΓΕΙΟ ΠΑΙΔΕΙΑΣ & ΘΡΗΣΚΕΥΜΑΤΩΝ, ΠΟΛΙΤΙΣΜΟΥ & ΑΘΛΗΤΙΣΜΟΥ
ΕΙΔΙΚΗ ΥΠΗΡΕΣΙΑ ΔΙΑΧΕΙΡΙΣΗΣ
Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης
ΕΣΠΑ 2007-2013
πρόγραμμα για την ανάπτυξη
ΕΥΡΩΠΑΪΚΟ ΚΟΙΝΩΝΙΚΟ ΤΑΜΕΙΟ

Η ΕΚΦΡΑΣΗ ΤΟΥ FOXP3 ΣΤΟ ΗΠΑΡ

ΣΧΕΤΙΖΕΤΑΙ ΜΕ ΤΟ ΒΑΘΜΟ ΑΛΛΑ ΟΧΙ

ΜΕ ΤΟ ΑΙΤΙΟ ΤΗΣ ΦΛΕΓΜΟΝΗΣ

Γ. Γερμανίδης, Ν. Αργέντου, Θ. Βασιλειάδης, Κ. Πατσιαούρα,
Κ.Μαντζούκης, Ε. Θεοχαρίδου, Π. Νικολαίδης, Α. Γερμενής, Μ. Σπελέτας

Regulatory T cells (Treg)

are an essential component of the immune system,

balancing necessary aggressiveness against foes

with tolerance for self-constituents

Sakaguchi S. *Annu Rev Immunol* 2004;22:531-562

Introduction_ Chronic Liver Diseases and Tregs

- Increased levels of Tregs in the blood of patients with chronic HBV infection

  Stoop et al, 2005

- Accumulation of Tregs in the liver of patients with chronic HBV infection

  Franzese et al, 2005

- Positive correlation between the HBV DNA level and the frequency of Tregs in the blood of chronically infected patients

  Stoop et al, 2007

- Higher frequency of $CD4^+CD25^+$ Tregs in peripheral blood of chronically HCV infected *vs* healthy/recovered persons

  Sugimoto et al, 2003; Cabrera et al, 2004

- Presence of $CD4^+FOXP3^+$ T cells in the liver of chronically HCV infected persons

  Scott et al, 2007

Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice

Jeong M Kim[1], Jeffrey P Rasmussen[1] & Alexander Y Rudensky[1,2]

Kim et al, *Nature Immunol* 2007;8: 191-197

- $CD4^{+}CD25^{+}$Foxp3 regulatory T Cells protect against T Cell-mediated fulminant hepatitis in a TGF-β-dependent manner in mice

Wei et al, 2008

Aim

Clarify the relation of liver Tregs (n,ind) to the expression of apoptosis mediators in the pathogenesis of liver damage (related to cause or intensity of inflammation?)

Analyzed Material_93/138 samples

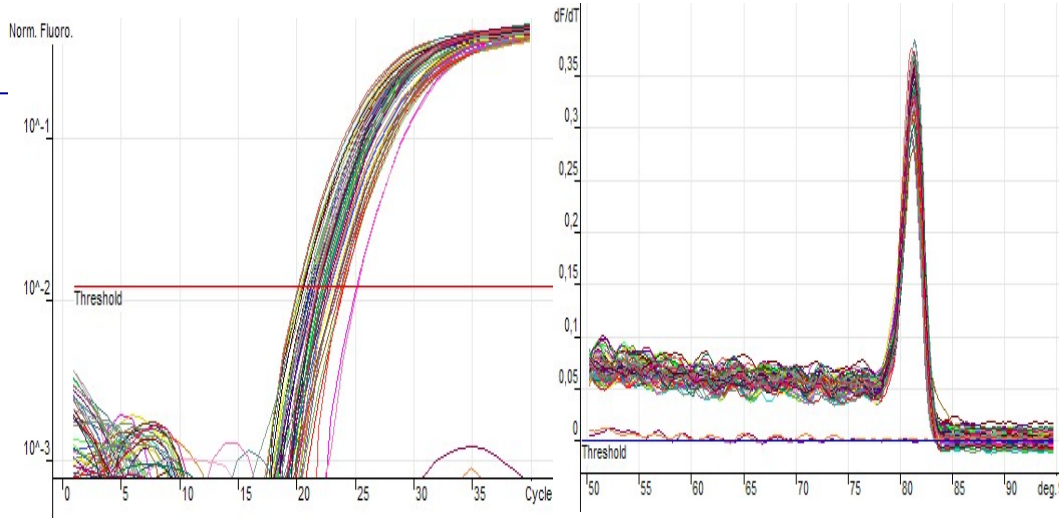
Patients with chronic liver diseases

- chronic HBV infection – 30 patients (15M/15F)
- chronic HBV infection after treatment/relapse- 7 patients (4M/3F)
- chronic HBV infection on LT treatment/remission- 12 patients (10M/2F)
- chronic HCV infection – 15 patients (12M/3F)
- NAFLD – 11 patients (6M/5F)
- Autoimmune hepatic diseases – 8 patients (4M/4F)
  - 4 patients with AIH (2M/2F)
  - 4 patients with PBC (4F)
- drug-induced hepatotoxicity (methotrexate) – 2 patients (0M/2F)

Normal controls (with minimal disease) – 8 subjects (5M/3F)

Liver biopsies obtained from 69 individuals (male/female: 56/37, mean age±SDEV: 47.6±12.47)

Study Design

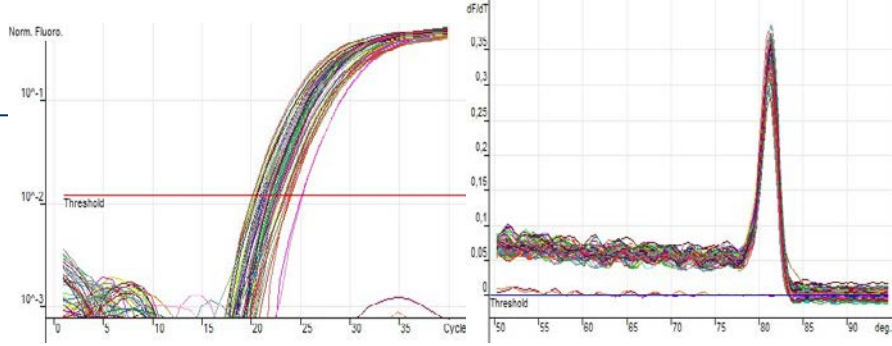


RNA → cDNA → Real Time PCR

Reference gene: b2M

Relative expression analysis: ΔΔCT method

Livak and Schmittgen, 2001



Relative expression analysis of…

FOXP3	Genes associated with Treg function
Fas/FasL, TRAIL	Mediators of apoptosis
Caspase 3	Effector molecules of apoptosis
IL-1b, IFN-γ, TNF-a	Inflammatory cytokines
IL-10, TGF-β1	Inducible Tregs

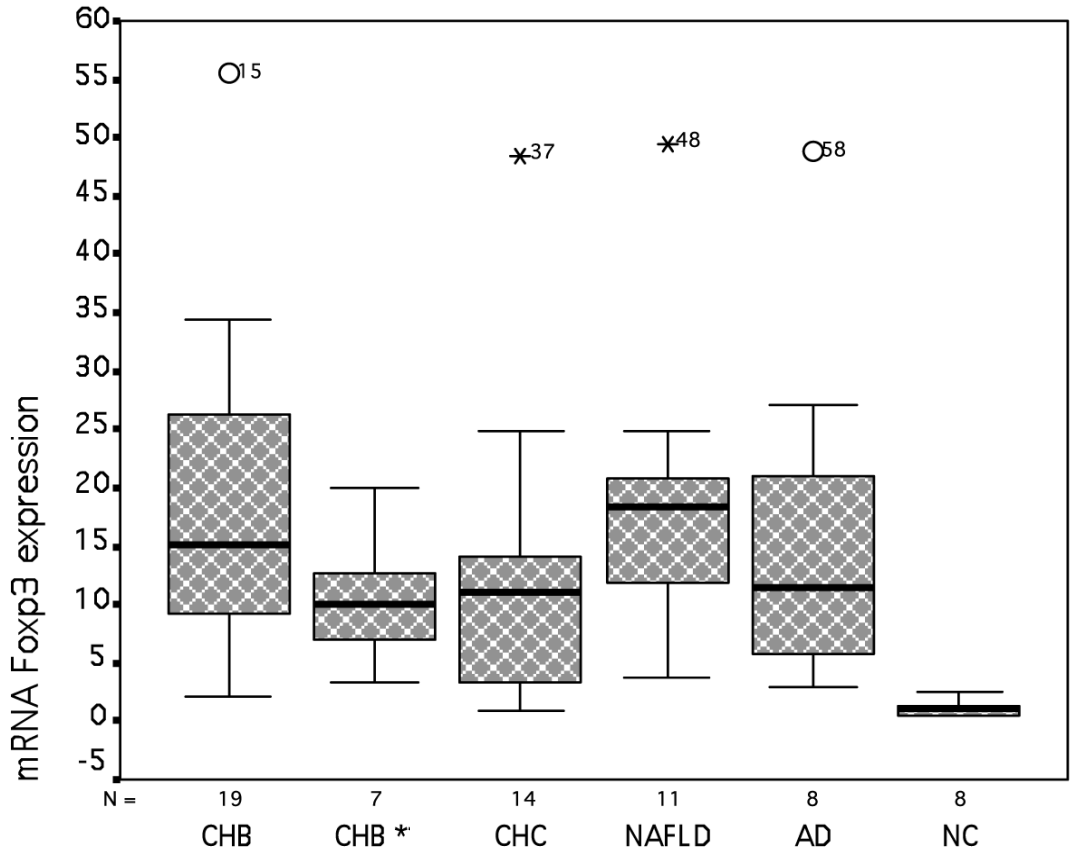
Results_ Gene expression in relation to liver diseases

Mann-Whitney U Test

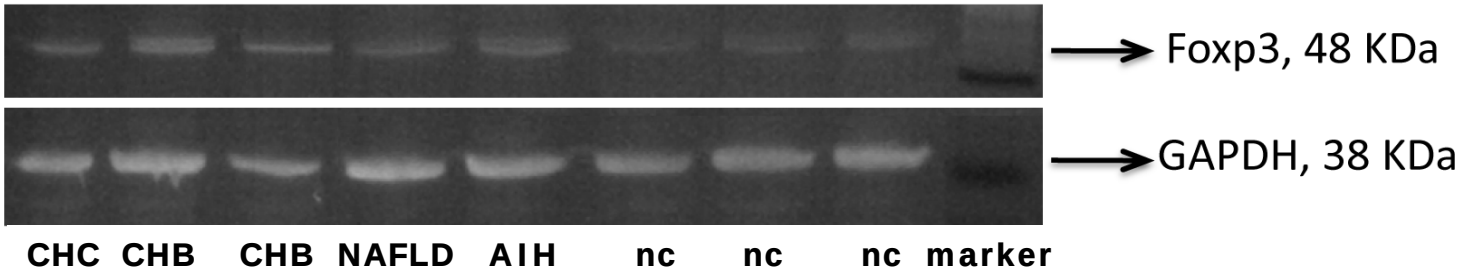
Gene	Normals (no 8)	Chronic HBV hepatitis				Chronic HCV hepatitis (no 14)		NAFLD [b] (no 11)		Autoimmune diseases [c] (no 8)		MTX-toxicity [d] (no 2)
		Diagnosis (no 19)		Treatment/Relapse (no 7)								
	mean±SD	mean±SD	p [a]	mean±SD	p [a]	mean±SD	p [a]	mean±SD	p [a]	mean±SD	p [a]	mean±SD
Foxp3	1.11±0.71	20.3±16.0	**<0.001**	10.4±5.47	**0.001**	12.7±12.3	**0.001**	18.4±11.9	**<0.001**	16.0±15.2	**0.001**	28.4±27.8
TGF-β1	1.56±1.49	1.10±0.84	0.710	0.53±0.31	0.053	0.73±0.58	0.065	1.60±1.01	0.620	1.43±1.04	0.834	0.72±0.17
IL-10	1.50±1.02	0.66±1.33	**0.011**	0.20±0.24	**0.016**	0.48±0.77	**0.017**	0.79±0.77	0.131	0.64±0.94	0.093	0.59±0.62
Fas	0.82±0.24	2.25±0.70	**<0.001**	2.12±0.78	**0.003**	1.55±1.07	0.179	3.51±1.17	**<0.001**	1.56±0.94	0.132	4.14±0.41
FasL	1.05±1.47	4.20±2.05	**0.001**	3.05±1.98	**0.028**	4.20±4.09	**0.004**	3.94±4.42	**0.004**	4.33±3.69	**0.008**	0.49±0.39
TRAIL	2.89±2.04	7.28±4.34	**0.007**	11.3±6.29	**0.015**	8.86±4.56	**0.001**	15.3±6.64	**<0.001**	3.52±2.11	0.355	15.3±12.7
Casp-3	1.87±1.85	1.13±0.74	0.915	0.76±0.43	0.366	1.66±1.34	0.700	1.97±1.05	0.283	3.19±2.23	0.093	2.28±0.66
TNF-α	3.43±4.70	1.77±3.12	0.307	1.54±1.93	0.201	2.73±3.58	0.544	8.17±11.0	0.480	6.63±9.11	0.186	1.21±0.93
IFN-γ	1.92±1.77	5.47±5.99	0.202	4.17±2.46	0.186	2.19±1.77	0.628	4.18±4.93	0.572	8.43±7.25	0.059	0.37±0.26
IL-1β	1.37±1.05	0.51±1.39	**0.022**	0.41±0.66	0.055	0.33±0.26	**0.042**	0.99±0.95	0.322	1.15±1.12	0.571	0.30±0.23

Results_ Gene expression in relation to liver diseases

A.

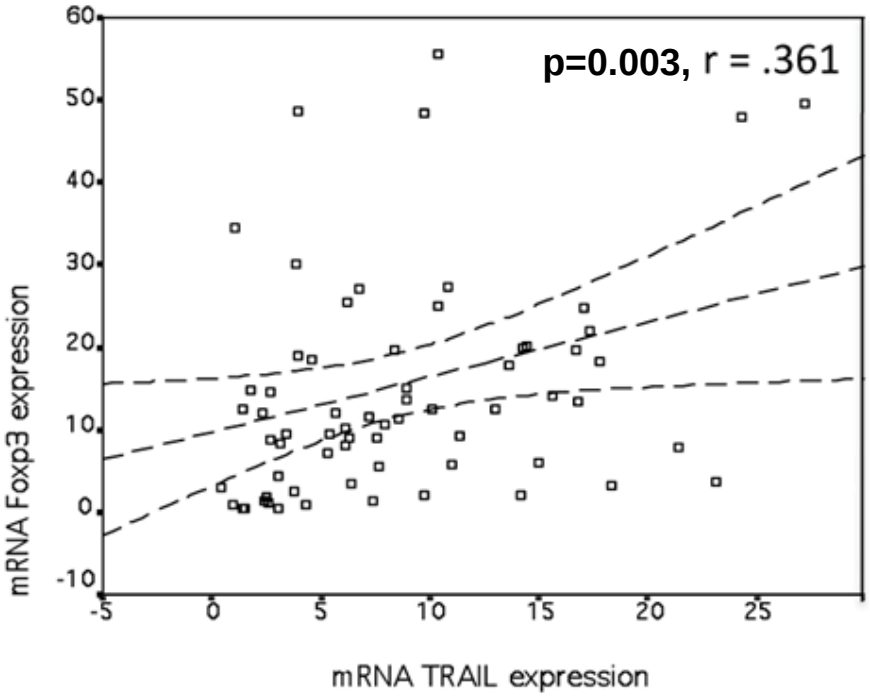


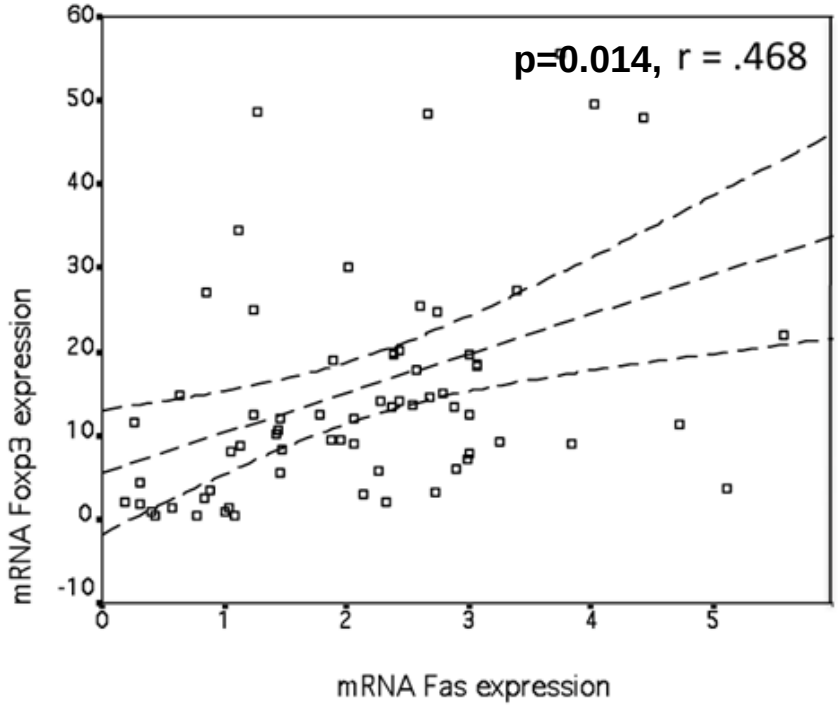
B.

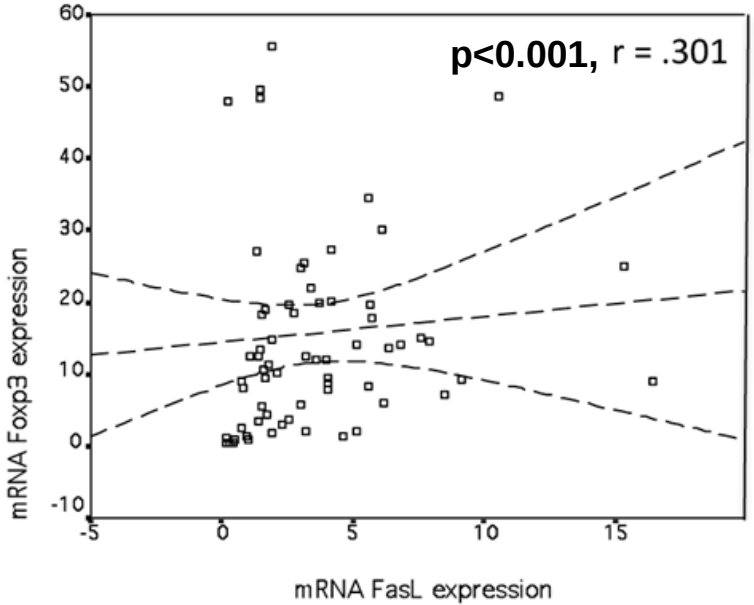


CHB* → chronic hepatitis B after treatment/relapse

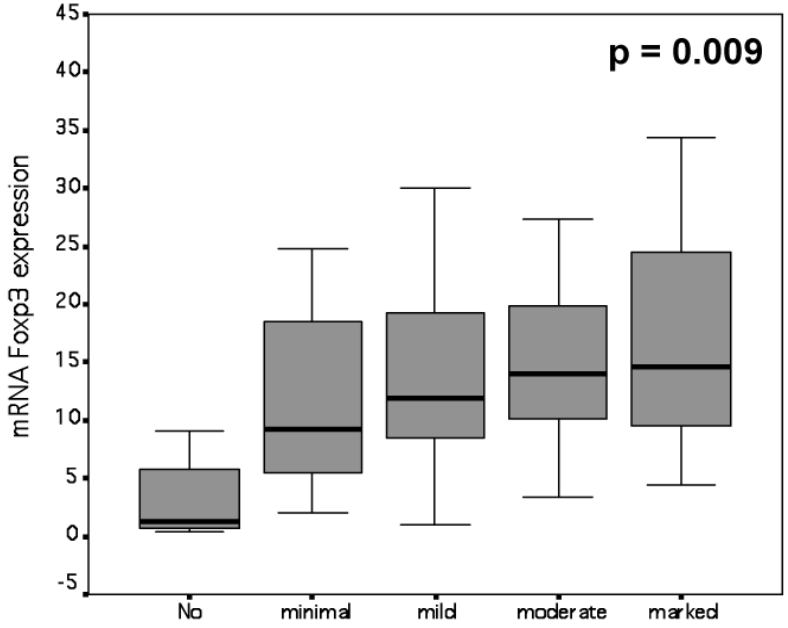
Results_ Correlation analysis between Foxp3 and apoptosis mediators

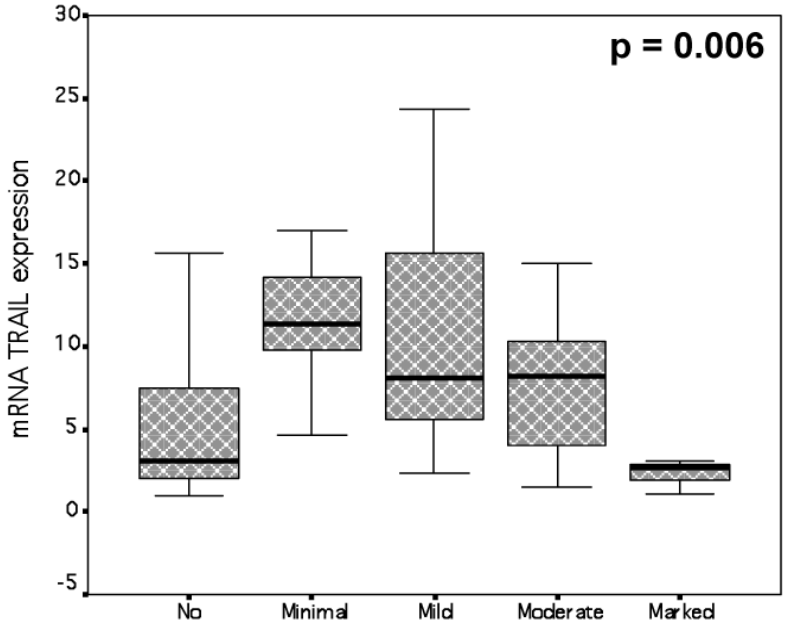


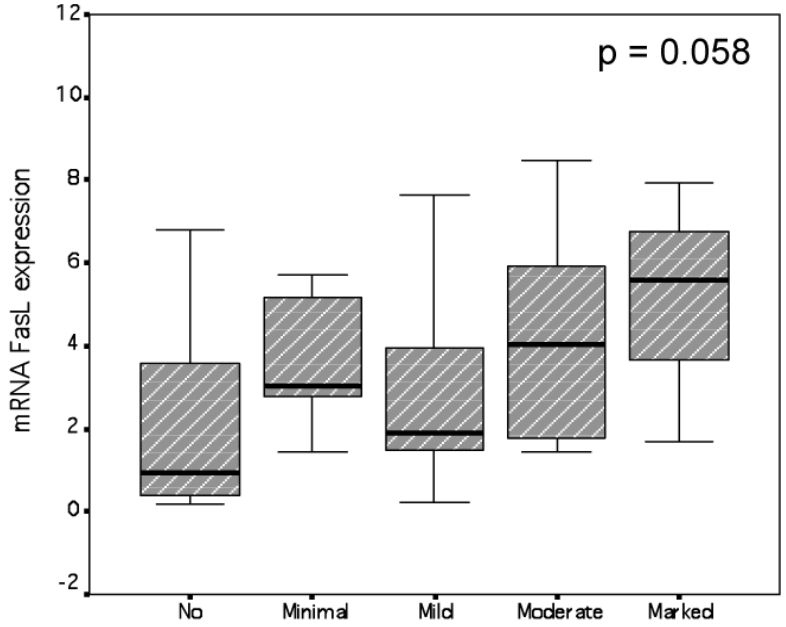


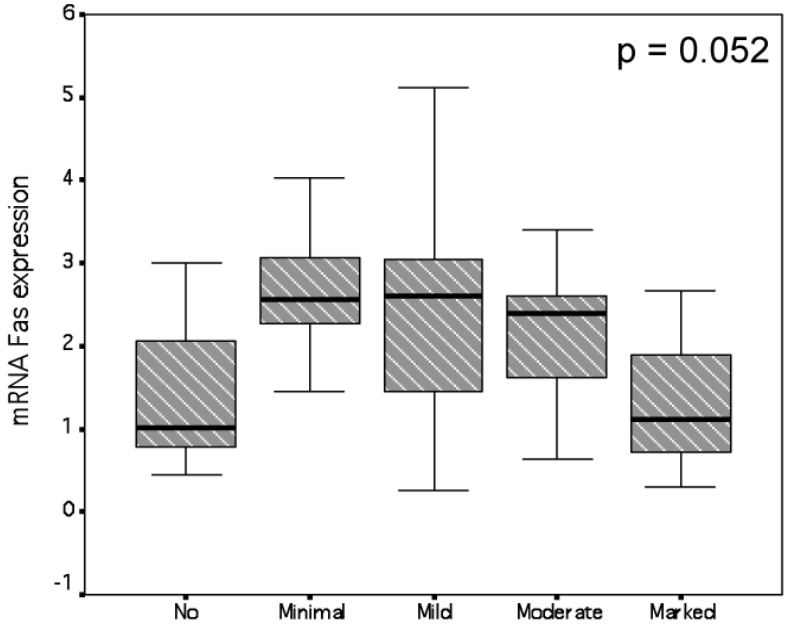


Results_ Gene expression in relation to liver inflammation









Hypothesis

↑ apoptosis

↓

↑ M phagocytosis

↓

↑self-antigen presentation by APCs
↑activation of autoreactive T cells

↓

expansion of nTregs (not iTregs)

↓

Prevention of catastrophic damage of self-tissues

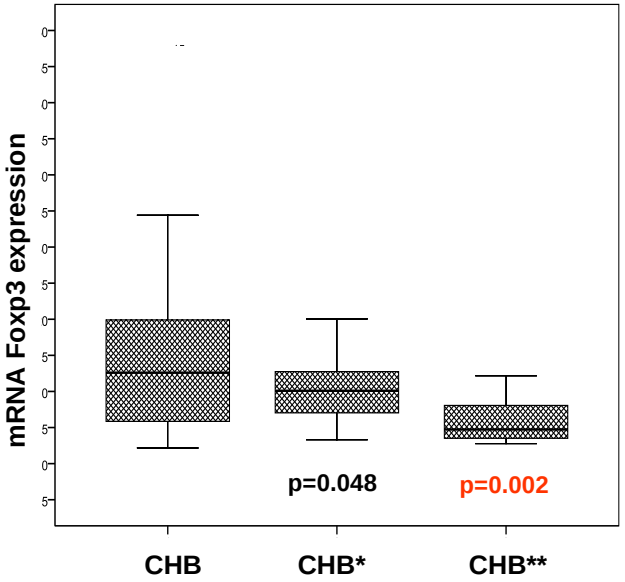
Suppression of virus-specific T cells (bystander?)

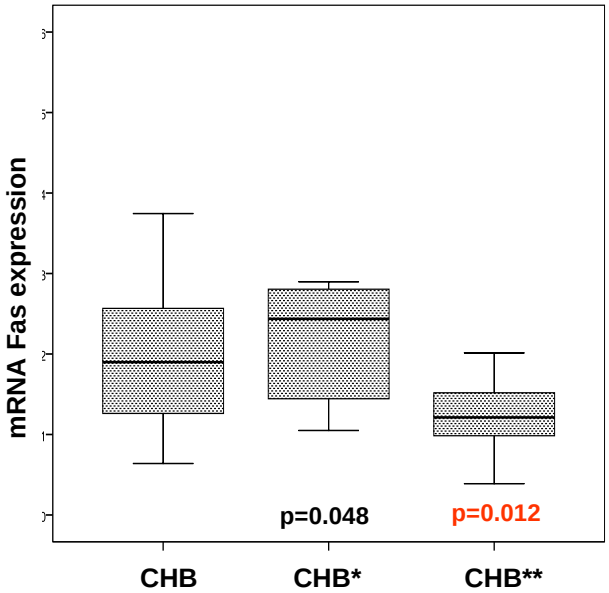
Results_ Gene expression in relation to CHB

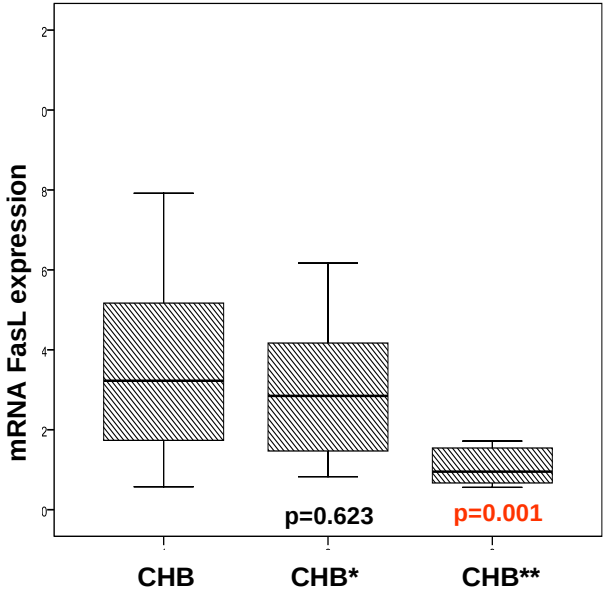
Gene	Chronic HBV hepatitis				
	Diagnosis (No 29)	Treatment / Relapse (No 7)		Treatment / Remission (No 12)	
	mean±SD	mean±SD	p [a]	mean±SD	p [a]
Foxp3	18.3±19.3	10.43±5.48	0.480	5.87±3.16	**0.002** ↓
IL-10	0.50±1.09	0.20±0.24	0.202	0.36±0.50	0.765
TGF-b1	0.87±0.75	0.52±0.32	0.187	0.69±0.45	0.543
Fas	1.98±0.84	2.13±0.77	0.480	1.23±0.49	**0.012** ↓
FasL	3.62±2.01	3.05±1.98	0.623	1.95±2.84	**0.001** ↓
TRAIL	7.80±4.19	11.3±6.3	0.165	12.±4.67	**0.012** ↑
IFN-g	5.95±5.47	4.18±2.46	0.856	4.08±6.47	0.079
IL-1b	0.37±1.12	0.41±0.66	0.741	0.14±0.14	0.192

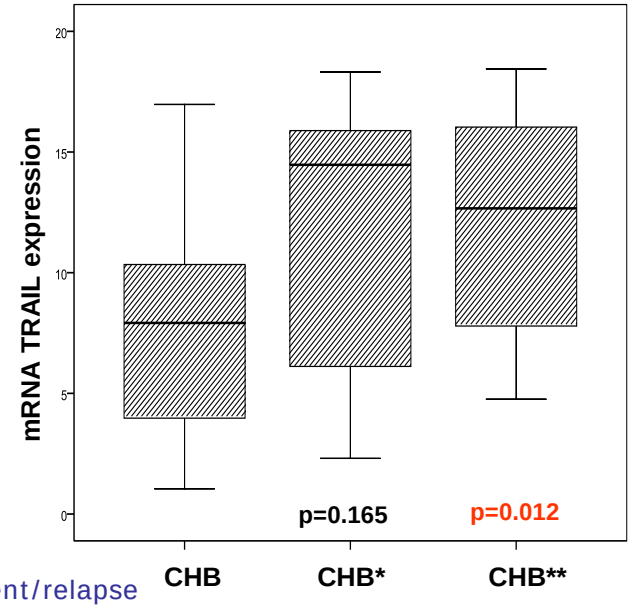
Mann-Whitney U Test

Results_ Gene expression in relation to CHB treatment/remission









CHB* → chronic hepatitis B after treatment/relapse

CHB** → chronic hepatitis B on long term treatment remission

Results_ Gene expression in relation to liver inflammation/fibrosis

- **TGF-b1** expression did not display any statistically significant change in relation to the intensity of inflammation

- Positive correlation between mRNA levels of **Foxp3** ($p<0.001$, $r=0.373$), **FasL** ($p<0.001$, $r=0.393$), and **IFN-g** ($p=0.003$, $r=0,319$) with fibrosis was observed

- Negative correlation between mRNA levels of **IL-10** ($p=0.002$, $r=-0.333$) and **Caspase-3** ($p=0.006$, $r=0.333$) with fibrosis was observed

- As expected, HAI score was strongly positively correlated with alanine aminotransferase (ALT) levels ($p=0.038$), in patients with CHB and CHC

Conclusions

- ✓ **Dramatic increase of liver quantitative Foxp3** expression in hepatic diseases that relates to the intensity of liver inflammation

- ✓ Significant **increase in mediators of apoptosis (Fas/FasL and TRAIL)** in viral hepatitis and NAFLD

- ✓ **CHB patients on long term treatment/remission (B,V,H) exhibit significant reduction of Fas/FasL and Foxp3 in the liver, compared to patients before treatment , or at relapse after Rx withdrawal**

- ✓ Decreased expression of IL-1β in viral hepatitis

Next Step

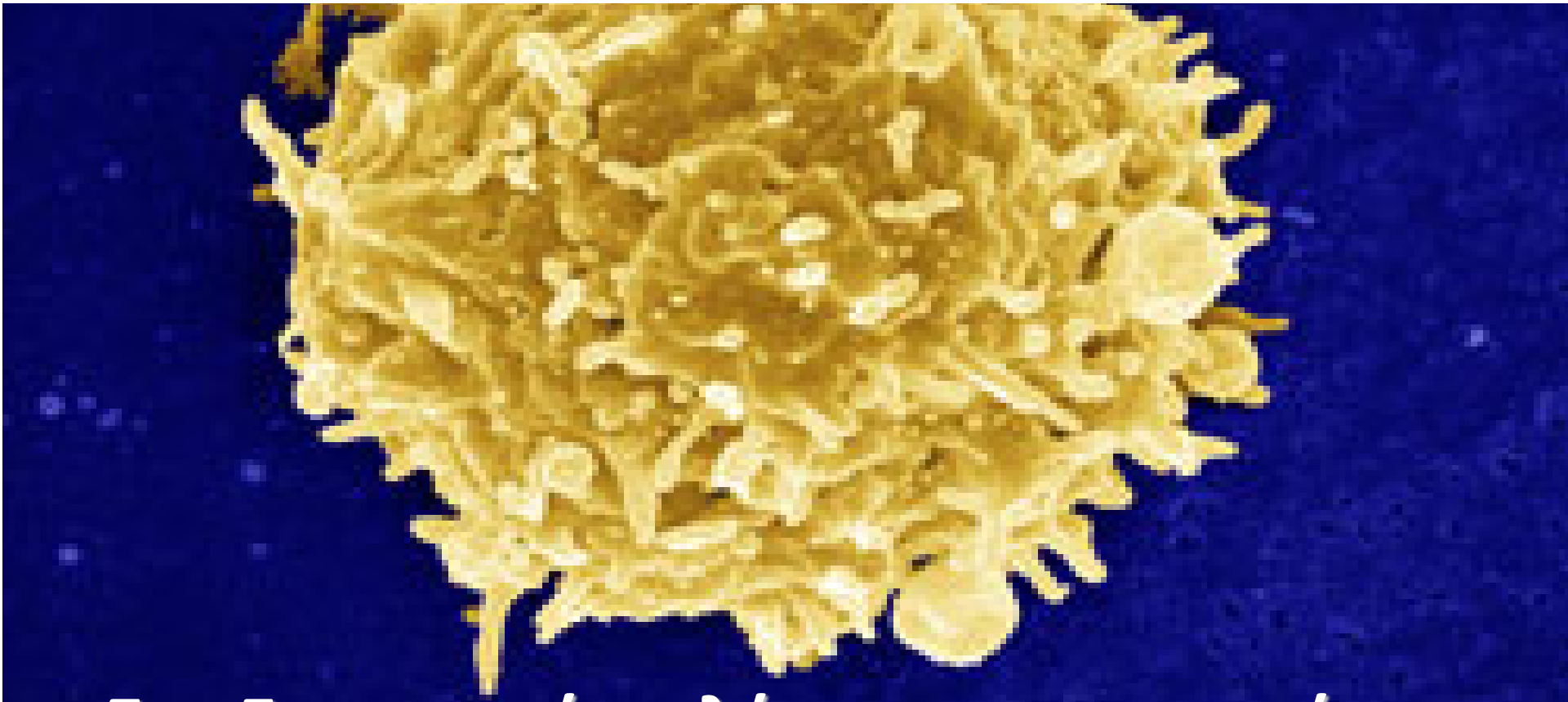
- Speletas M., et al., Foxp3 expression in liver correlates with the degree but not the cause of inflammation , Mediators of Inflammation 2011; in press
- Funded by the Hellenic Association for the study of the Liver
- Funded by the Basic Research Programme Herakleitos (2007-13)
- Presented in part: AASLD 2008, EASL 2010
- Increase the number of biopsies on LT treatment/remission and after treatment/relapse of patients with CHB
- Analysis of molecules related to autophagy and PD-1/PD L-1,2

Ευρωπαϊκή Ένωση
Ευρωπαϊκό Κοινωνικό Ταμείο

ΕΠΙΧΕΙΡΗΣΙΑΚΟ ΠΡΟΓΡΑΜΜΑ
ΕΚΠΑΙΔΕΥΣΗ ΚΑΙ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗ
επένδυση στην κοινωνία της γνώσης
ΥΠΟΥΡΓΕΙΟ ΠΑΙΔΕΙΑΣ & ΘΡΗΣΚΕΥΜΑΤΩΝ, ΠΟΛΙΤΙΣΜΟΥ & ΑΘΛΗΤΙΣΜΟΥ
ΕΙΔΙΚΗ ΥΠΗΡΕΣΙΑ ΔΙΑΧΕΙΡΙΣΗΣ

ΕΣΠΑ 2007-2013
πρόγραμμα για την ανάπτυξη
ΕΥΡΩΠΑΪΚΟ ΚΟΙΝΩΝΙΚΟ ΤΑΜΕΙΟ

Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης



Σας Ευχαριστώ πολύ για την προσοχή σας